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Patentavdelningen



SE001 A67

PCT/SE 00/01767

10/070412

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|-------------------|
| REC'D 01 NOV 2000 |
| WIPO PCT |

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4

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(21) Patentansökningsnummer 9903336-7
Patent application number

(86) Ingivningsdatum 1999-09-17
Date of filing

Stockholm, 2000-10-20

För Patent- och registreringsverket
For the Patent- and Registration Office

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Fee

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29295/BN

DNA construct and its use.

The present invention relates to a new DNA construct for transformation into oilseed plants. The DNA construct comprises nucleotide sequences encoding peptides with enzyme activities necessary for the high-level production and esterification of keto group-containing xanthophylls in oilseed plants.

Background of the invention

Carotenoids are produced *de novo* by plants, fungi, algae and some bacteria. A number of biosynthetic steps are needed for the biological production of the carotenoids. There are two chemically different groups of carotenoids, namely carotenes containing only carbon and hydrogen molecules and xanthophylls containing oxygen in the molecule in addition to carbon and hydrogen.

The xanthophylls, and particularly astaxanthin (3,3'-dihydroxy- β - β -carotene-4,4'-dione), are often colored pigments and are used as such or as anti-oxidants.

Carotenes are biological precursors for the production of the oxygen-containing xanthophylls. There are two types of enzymes responsible for the introduction of hydroxy groups and keto groups into the carotenes, namely hydroxylases and ketolases, respectively.

The keto group-containing xanthophyll astaxanthin, which has keto and hydroxy groups, is biosynthetically produced from beta-carotene.

Large-scale production of xanthophylls from natural sources is at present performed by AstaCarotene AB, Gustavsberg, Sweden, by cultivation of the alga *Haematococcus pluvialis* for the production of astaxanthin in esterified form.

It would be desirable to be able to produce keto group-containing xanthophylls particularly astaxanthin, in oilseed plants. Oilseed plants have naturally β -carotene hydroxylases but lack β -carotene C-4-oxygenase enzymes or ketolases.

Description of the invention

The present invention provides DNA constructs enabling and promoting production of keto group containing xanthophylls, especially astaxanthin, in oilseed plants, such as rape, sunflower, soybean and mustard. The DNA construct is transformed into the oilseed plant cell for expression of a protein or fused protein which has an enzyme activity enabling keto group insertion into a carotene or hydroxy carotene for the biosynthetic production of a keto group containing xanthophyll, such as cantaxanthin (β , β -carotene-4,4'-dione) and/or astaxanthin. Use is thus made of the biosynthetic pathway of the oilseed plant to

produce carotenoids. The naturally occurring synthesis of carotenoids involves a number of enzymes, namely 1-D-deoxyxylulose 5-phosphate synthase, isopentenyl pyrophosphate:dimethylallyl pyrophosphate isomerase, geranylgeranyl pyrophosphate synthase, phytoene synthase, phytoene desaturase, zeta-carotene desaturase, lycopene beta-cyclase, β -carotene hydroxylase, and β -carotene C-4-oxygenase. Genes coding for peptides having these enzymatic activities may be inserted into the DNA construct of the invention, one or several per construct, to promote high-level production in the transgenic oilseed plant. In case only one enzyme coding gene is inserted per plant, two or more plants may be sexually interbred to produce plants containing all the desired enzyme activities.

10 Thus, the present invention is directed to a DNA construct comprising in the 5' to 3' direction of transcription operably linked a promoter region directing transcription to the seed of an oilseed plant, a nucleotide sequence coding for at least one peptide with enzyme activity necessary for keto group containing xanthophyll production and esterification in an oilseed plant and a transcriptional termination region.

15 In a preferred embodiment of the invention the DNA construct additionally comprises between the promoter region and the nucleotide sequence coding for at least one peptide with enzyme activity a nucleotide sequence coding for a transit peptide directing the translated fusion polypeptide to the chloroplast of the oilseed plant.

20 The DNA construct is preferably such that the promoter is a napin promoter, the peptide with enzyme activity necessary for keto group containing xanthophyll production is selected from the group consisting of peptides with 1-D-deoxyxylulose 5-phosphate synthase, isopentenyl pyrophosphate:dimethylallyl pyrophosphate isomerase, geranylgeranyl pyrophosphate synthase, phytoene synthase, phytoene desaturase, zeta-carotene desaturase, lycopene beta-cyclase, β -carotene hydroxylase, and β -carotene C-4-oxygenase activity. To 25 promote esterification of astaxanthin a nucleotide sequence coding for a peptide with acyl transferase activity may be included in the group.

25 In a preferred embodiment of the DNA construct according to the invention the nucleotide sequence coding for a peptide with enzyme activity is a nucleotide sequence coding for a N-terminally truncated β -carotene C-4-oxygenase gene from the alga 30 *Haematococcus pluvialis*.

An example of the DNA construct of the invention is presented in the sequence listing as SEQ ID NO:1 and in Fig.1.

The present invention is also directed to a transgenic oilseed plant cell comprising the DNA construct of the invention, and preferably the oilseed plant is selected from the group consisting of rape, sunflower, soybean and mustard.

The invention is additionally directed to transgenic oilseed plant-produced xanthophyll, e.g. canthaxanthin and astaxanthin.

A preferred aspect of the invention is directed to transgenic oilseed plant-produced astaxanthin esters.

The present invention will now be illustrated with reference to the DNA construct disclosed in the sequence listing and in Fig.1, and the following description of embodiments. However, the invention is not limited to these exemplifications.

Short description of the drawings

Fig.1 illustrates the nucleotide sequence of the DNA construct comprising the napin promoter, the chloroplast localization signal, the N-terminally truncated β -carotene C-4-oxygenase gene and the termination sequence, and the deduced amino acid sequences of the transit peptide and the β -carotene C-4-oxygenase.

Description of embodiments

The invention is illustrated by production of astaxanthin in the seed of oilseed rape. The astaxanthin produced in the seed of the transgenic plant is extracted as part of the extracted oil. By use of conventionally used protocols for *Agrobacterium tumefaciens* mediated transformation such as described by (Hoekema et al. 1983, An et al. 1986, Fry et al. 1987, DeBlock et al. 1988, Radke et al. 1988, or Moloney et al. 1989) transgenic plants are produced having a chimeric DNA construct that is genetically inherited and is able to produce astaxanthin. The nucleotide sequence of the chimeric DNA construct consist of four parts of different genetic origin namely: (1) a promoter, (2) a localization signal, (3) a β -carotene C-4-oxygenase coding region and (4) a termination sequence.

The napin promoter directs transcription to the seed of oilseed rape (Stålberg et al 1996). This promoter was coupled to a localization signal similar but not identical to a transit peptide (TP) of *Rbcs1a* (Krebbers, 1988) that directs the translated product of a fused gene to the chloroplast. The promoter and the TP sequence were ligated to a part of the coding sequence of a ketolase gene BCK (Kajiwara et al. 1995). This enzyme oxygenates β -carotene to canthaxanthin, (Fraser et al. 1997). The chimeric DNA construct was then coupled to a suitable termination sequence, e.g. that of the *Agrobacterium tumefaciens* nopaline synthase gene (the nos 3' end)(Bevan et al. 1983), as illustrated in Fig.1.

Cellular storage of Astaxanthin

The storage of large amounts of free astaxanthin in plants will be difficult due to toxic effects of the molecule as it intercalates in the plant membranes. An effective esterification of astaxanthin to fatty acids enables storage of the esterified molecules in triacylglycerol containing oleosomes. Thus, an acyl transferase can be claimed to be of fundamental importance for the process, as is proteins that can mediate transport of different forms of astaxanthin from the chloroplast to the vesicles.

Sequences and oligonucleotides used in the construction of the DNA construct*1. Napin promoter (GeneBank ACCESSION No. J02798)*

10 This promoter sequence, a 1145 base pair fragment including the 5' leader sequence has a unique HindIII site at the 5' end. The 3' end was synthesized with an additionally 6 nucleotide BamHI site.

2. Transit peptide similar to RBCS1a (GeneBank ACCESSION No. X13611, X14565)

15 The transit peptide (TP) was amplified by PCR from -28 to the end of the transit cleavage aa=54/55 site of the Rbcs1a gene. The 5' end was synthesized with a BamHI site and similarly the 3' sequence was synthesized with a XbaI site. The two following oligonucleotides were used for the PCR amplification.

BamHI

20 5' primer: TP1 5'AGAC **GGATCC** TCAGTCACACAAAGAGTA 3'

SacI XbaI

3' primer: TP2 5'GTTC **GAGCTC** TCTAGA CATGCAGTTAACGC 3'

3. BCK (β -carotene C-4 oxygenase) (Genebank ACCESSION No. D45881)

25 The BCK fragment was amplified by PCR including a 5' XbaI site and was ligated to the TP already described. The 5' primer (BCK1) used for PCR, is homologous to the BCK sequence from nucleotide 264 and the 3' oligonucleotide (Ax40) ends with a stop codon and was synthesized with a SacI restriction site for cloning. The synthesized fragment was fused to the TP as shown in Fig 1.

30 Oligonucleotides used for PCR:

XbaI

5' primer: BCK1 5'ACAG **TCTAGA** ATGCCATCCGAGTCGTCA 3'

SacI

3' primer: AX40 5'CACCGAGCTCCATGACACTCTTGTGCAGA 3'

Description of SEQ ID NO:1 and SEQ ID NO:2

The sequences shown in Fig.1 are the same as the two sequences which are shown in the sequence listing.

The SEQ ID NO:1 is a nucleotide sequence composed of the following features:

5

Nucleotide No.

| | | |
|----|---|-----------|
| | Cloning site HindIII | 1-6 |
| | Napin Promoter | 1-1145 |
| | Cloning site BamHI | 1146-1151 |
| | Transit peptide leader | 1152-1178 |
| 10 | Transit peptide coding | 1179-1347 |
| | Cloning site XbaI | 1348-1353 |
| | β -carotene C-4-oxygenase | 1354-2217 |
| | β -carotene C-4-oxygenase 3' untranslated | 2218-2266 |
| | Cloning site SacI | 2267-2272 |
| 15 | Nopaline synthetase termination | 2273-2536 |
| | Cloning site EcoRI | 2538-2543 |

The SEQ ID NO: 2 is a deduced amino acid sequence of the fusion protein of the transit peptide and the peptide with β -carotene C-4-oxygenase activity.

References

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Fry J, Barnason A, and Horsch RB, (1987). Transformation of *Brassica napus* with *Agrobacterium tumefaciens* based vectors. *Plant Cell Reports* 6:321-325.

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Kajiwara S, Kakizono T, Saito T, Kondo K, Ohtani T, Nishio N, Nagai S and Misawa N. (1995). Isolation and functional identification of a novel cDNA for astaxanthin biosynthesis from *Haematococcus pluvialis*, and astaxanthin synthesis in *Escherichia coli* *Plant Mol. Biol.* 30 29 (2), 343-352.

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Pua E-C, Mehra-Palta A, Nagy F and Chua N-H, (1987). Transgenic plants of *Brassica napus*. Biotechnology vol 5, 815-817.

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SEQUENCE LISTING

<110> AstaCarotene AB

<120> DNA construct and its use

<130> 29295-AstaCarotene

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<141>

<160> 2

<170> PatentIn Ver. 2.1

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<211> 2543

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<213> Artificial Sequence

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<223> Description of Artificial Sequence: napin promoter
+ chloroplast localization signal + beta-carotene C-4 oxygenase
coding sequence + termination sequence

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<221> promoter

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<221> terminator

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tttccaaacat tttaaatttc actattggct gaatgcttct tctttgagga agaaacaatt 180
cagatggcag aaatgtatca accaatgcat atatacaaatt gtacctcttg ttctcaaaac 240
atctatcgga tggttccatt tgctttgtca tccaaattgt gactacttta tattattcac 300
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aactcaaatt cgattgacat gtatccattc aacataaaat taaaccagcc tgcacctgca 600
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 Met Ala Ser Ser Met
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 Leu Ser Ser Ala Thr Met Val Ala Ser Pro Ala Gln Ala Thr Met Val
 10 15 20

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 Ala Pro Phe Asn Gly Leu Lys Ser Ser Ala Ala Phe Pro Ala Thr Arg
 25 30 35

aag gct aac aac gac att act tcc atc aca agc aac ggc gga cgc gtt 1338
 Lys Ala Asn Asn Asp Ile Thr Ser Ile Thr Ser Asn Gly Gly Arg Val
 40 45 50

aac tgc atg tct aga atg cca tcc gag tcc tca gac gca gct cgt cct 1386
 Asn Cys Met Ser Arg Met Pro Ser Glu Ser Ser Asp Ala Ala Arg Pro
 55 60 65

gcg cta aag cac gcc tac aaa cct cca gca tct gac gcc aag ggc atc 1434
 Ala Leu Lys His Ala Tyr Lys Pro Pro Ala Ser Asp Ala Lys Gly Ile
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 Gly Leu Phe Ile Thr Thr His Asp Ala Met His Gly Thr Ile Ala Leu
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 Arg His Arg Gln Leu Asn Asp Leu Leu Gly Asn Ile Cys Ile Ser Leu
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 Tyr Ala Trp Phe Asp Tyr Ser Met Leu His Arg Lys His Trp Glu His
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 Ser Leu Trp Gln Phe Ala Arg Leu Ala Trp Trp Ala Val Val Met Gln
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 Met Leu Gly Ala Pro Met Ala Asn Leu Leu Val Phe Met Ala Ala Ala
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 Pro Ile Leu Ser Ala Phe Arg Leu Phe Tyr Phe Gly Thr Tyr Leu Pro
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 Phe Arg Ala Lys Thr Ser Glu Ala Ser Asp Val Met Ser Phe Leu Thr
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 tgc tac cac ttt gac ctg cac tgg gag cac cac aga tgg ccc ttt gcc 2154
 Cys Tyr His Phe Asp Leu His Trp Glu His His Arg Trp Pro Phe Ala
 310 315 320 325

 ccc tgg tgg cag ctg ccc cac tgc cgc cgc ctg tcc ggg cgt ggc ctg 2202
 Pro Trp Trp Gln Leu Pro His Cys Arg Arg Leu Ser Gly Arg Gly Leu
 330 335 340

 gtg cct gcc ttg gca tgacctggtc cctccgctgg tgacccagcg tctgcacaag 2257
 Val Pro Ala Leu Ala
 345

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<213> Artificial Sequence

<223> Description of Artificial Sequence: deduced fusion protein of
transit peptide + peptide with beta-carotene C-4 oxygenase activity

<400> 2

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1 5 10 15

Gln Ala Thr Met Val Ala Pro Phe Asn Gly Leu Lys Ser Ser Ala Ala
20 25 30

Phe Pro Ala Thr Arg Lys Ala Asn Asn Asp Ile Thr Ser Ile Thr Ser
35 40 45

Asn Gly Gly Arg Val Asn Cys Met Ser Arg Met Pro Ser Glu Ser Ser
50 55 60

Asp Ala Ala Arg Pro Ala Leu Lys His Ala Tyr Lys Pro Pro Ala Ser
65 70 75 80

Asp Ala Lys Gly Ile Thr Met Ala Leu Thr Ile Ile Gly Thr Trp Thr
85 90 95

Ala Val Phe Leu His Ala Ile Phe Gln Ile Arg Leu Pro Thr Ser Met
100 105 110

Asp Gln Leu His Trp Leu Pro Val Ser Glu Ala Thr Ala Gln Leu Leu
115 120 125

Gly Gly Ser Ser Ser Leu Leu His Ile Ala Ala Val Phe Ile Val Leu
130 135 140

Glu Phe Leu Tyr Thr Gly Leu Phe Ile Thr Thr His Asp Ala Met His
145 150 155 160

Gly Thr Ile Ala Leu Arg His Arg Gln Leu Asn Asp Leu Leu Gly Asn
165 170 175

Ile Cys Ile Ser Leu Tyr Ala Trp Phe Asp Tyr Ser Met Leu His Arg
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Lys His Trp Glu His His Asn His Thr Gly Glu Val Gly Lys Asp Pro
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Asp Phe His Lys Gly Asn Pro Gly Leu Val Pro Trp Phe Ala Ser Phe
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Met Ser Ser Tyr Met Ser Leu Trp Gln Phe Ala Arg Leu Ala Trp Trp
225 230 235 240

Ala Val Val Met Gln Met Leu Gly Ala Pro Met Ala Asn Leu Leu Val
245 250 255

Phe Met Ala Ala Ala Pro Ile L u Ser Ala Phe Arg Leu Phe Tyr Phe
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Gly Thr Tyr Leu Pro His Lys Pro Glu Pr Gly Pro Ala Ala Gly Ser
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Gln Val Met Ala Trp Phe Arg Ala Lys Thr Ser Glu Ala Ser Asp Val
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Met Ser Phe Leu Thr Cys Tyr His Phe Asp Leu His Trp Glu His His
305 310 315 320

Arg Trp Pro Phe Ala Pro Trp Trp Gln Leu Pro His Cys Arg Arg Leu
325 330 335

Ser Gly Arg Gly Leu Val Pro Ala Leu Ala
340 345

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Claims

1. A DNA construct comprising in the 5' to 3' direction of transcription operably linked a promoter region directing transcription to the seed of an oilseed plant, a nucleotide sequence coding for at least one peptide with enzyme activity necessary for keto group containing xanthophyll production and esterification in an oilseed plant and a transcriptional termination region.
5
2. The DNA construct according to claim 1, which between the promoter region and the nucleotide sequence coding for at least one peptide with enzyme activity additionally comprises a nucleotide sequence coding for a transit peptide directing the translated fusion polypeptide to the chloroplast of the oilseed plant.
10
3. The DNA construct according to claim 1 or 2, wherein the promoter is a napin promoter, the peptide with enzyme activity necessary for keto group containing xanthophyll production and esterification is selected from the group consisting of peptides with, 1-D-
15 deoxyxylulose 5-phosphate synthase, isopentenyl pyrophosphate:dimethylallyl pyrophosphate isomerase, geranylgeranyl pyrophosphate synthase, phytoene synthase, phytoene desaturase, zeta-carotene desaturase, lycopene beta-cyclase, β -carotene hydroxylase, β -carotene C-4-oxygenase, and acyl transferase activity.
4. The DNA construct according to any one of claims 1 - 3, wherein the
20 nucleotide sequence coding for a peptide with enzyme activity is a nucleotide sequence coding for a N-terminally truncated β -carotene C-4-oxygenase gene from the alga *Haematococcus pluvialis*.
5. The DNA construct according to claim 4, wherein the nucleotide sequence is SEQ ID NO:1.
- 25 6. Transgenic oilseed plant cell comprising the DNA construct of any one of claims 1-5 .
7. Transgenic oilseed plant cell according to claim 6, wherein the oilseed plant is selected from the group consisting of rape, sunflower, soybean and mustard.
- 30 8. Transgenic oilseed plant-produced xanthophyll.
9. Transgenic oilseed plant-produced canthaxanthin
10. Transgenic oilseed plant-produced astaxanthin.
11. Transgenic oilseed plant-produced astaxanthin esters.

Napin promoter

AAGCTTTCTTCATCGGTGATTGATTCTTAAAGACTTATGTTCTTATCTTGCTTCTGA
 GGCAAGTATTCAAGTACCAAGTTACCACTTATATTCTGGACTTCTGACTGCATCCTCATT
 TTTCCAACATTTAAATTCACATTGGCTGAATGCTTCTTGTGAGGAAGAACAAATT
 CAGATGGCAGAAATGTATCAACCAATGCATATATAACAAATGTACCTCTGTTCTCAAAAC
 ATCTATCGGATGGTTCCATTGCTTGTCAATTAGTGACTIONTATATTATTAC
 TCCTCTTATTACTATTTCATGCGAGGTTGCCATGTACATTATATTGTAAGGATTGAC
 GCTATTGAGCGTTTCTTCAATTCTTATTAGACATGGTATGAAATGTGTGTTA
 GAGTTGGTTGAATGAGATATACGTTCAAGTGAAGTGGCATACCGTTCTCGAGTAAGGAT
 GACCTACCCATTCTTGAGACAAATGTTACATTAGTATCAGAGTAAATGTGTACCTAT
 AACTCAAATTGATTGACATGTATCCATTCAACATAAAATTAAACCAGCCTGCACCTGCA
 TCCACATTCAAGTATTCAAAACGTTGGCTCCTATCCACCGGGTGTAAACAGACGGA
 TTCCGAATTGGAAGATTTGACTCAAATTCCAATTATATTGACCGTGACTAAATCAA
 CTTTAACCTCTATAATTCTGATTAAGCTCCAAATTATATTCCAACGGCACTACCTCCA
 AAATTATAGACTCTCATCCCCTTAAACCAACTTAGTAAACGTTTTTTTTAATT
 TATGAAGTTAAGTTTACCTTGTAAAAAGAATGTTCAAGATGCCATGCCAGA
 ACATTAGCTACACGTTACACATAGCATGCAGCCGGAGAATTGTTTCTCGCCACTT
 GTCACCCCTCAAACACCTAACAGAGCTCTCTCACAGCACACACATAACATGC
 GTGCATGCATTATTACACGTATGCCATGCAAATCTCCTTATAGCCTATAAATTAACT
 CATCCGCTTCACTCTTACTCAAACCAAAACTCATCAATAACAAAGATTAAAAACATA

End -28 untranslated leader TP start
 CACGAGGATCCTCAGTCACACAAAGAGTAAAGAAGAACATGGCTCCTCTATGCTCT
 M A S S M L S
 TCCGCTACTATGGTTGCCTCTCGGCTCAGGCCACTATGGTCGCTCCTTCAACGGACTT
 S A T M V A S P A Q A T M V A P F N G L
 AAGTCCTCCGCTGCCTTCCCAGCCACCCGAAGGCTAACACGACATTACTCCATCACA
 K S S A A F P A T R K A N N D I T S I T

FIG.1

TP End C-4-Oxygenase

AGCAACGGCGGACGCGTTAAC TGCA TGCTAGAATGCCATCCGAGTCGT CAGACGCAGCT
 S N G G R V N C M S R M P S E S S D A A

 CGTCCTGCGCTAAAGCACGCCTACAAACCTCCAGCATCTGACGCCAAGGGCATCACGATG
 R P A L K H A Y K P P A S D A K G I T M

 GCGCTGACC ATCATTGGCACCTGGACCGCAGTGT TACACGCAATATTCAAATCAGG
 A L T I I G T W T A V F L H A I F Q I R

 CTACCGACATCCATGGACCA GCTTCACTGGTTGCC TGTGTCCGAAGCCACAGCCCAGCTT
 L P T S M D Q L H W L P V S E A T A Q L

 TTGGGCGGAAGCAGCAGCCTACTGCACATCGCTGCAGTCTTCATTGACTTGAGTTCTG
 L G G S S S L L H I A A V F I V L E F L

 TACACTGGTCTATTCATCACCA CACATGACGCAATGCATGGCACCA TAGCTTGAGGCAC
 Y T G L F I T T H D A M H G T I A L R H

 AGGCAGCTCAATGATCTCCTGGCAACATCTGCATATCACTGTACGCC TGGTTGACTAC
 R Q L N D L L G N I C I S L Y A W F D Y

 AGCATGCTGCATCGCAAGCACTGGGAGCACCACACCATACTGGCGAAGTGGGAAAGAC
 S M L H R K H W E H H N H T G E V G K D

 CCTGACTTCCACAAGGGAAATCCCGGCCTGTCCCTGGTTCGCCAGCTTCATGTCCAGC
 P D F H K G N P G L V P W F A S F M S S

 TACATGTCCCTGTGGCAGTTGCCCGGCTGGCATGGTGGCAGTGGT GATGCAAATGCTG
 Y M S L W Q F A R L A W W A V V M Q M L

 GGGCGCCCATGGCAAATCTCCTAGTCTTCATGGCTGCAGCCC AATCTGT CAGCATT C
 G A P M A N L L V F M A A A P I L S A F

 CGCCTCTTCTACTCGGCACTTACCTGCCACACAAGCCTGAGCCAGGCC TGCAGCAGGC
 R L F Y F G T Y L P H K P E P G P A A G

 TCTCAGGTGATGGCCTGGT CAGGGCCAAGACAAGTGAGGCATCTGATGT GATGAGTTTC
 S Q V M A W F R A K T S E A S D V M S F

 CTGACATGCTACCACTTGACCTGC ACTGGGAGCACCACAGATGCCCTTGCCCCCTGG
 L T C Y H F D L H W E H H R W P F A P W

C-4 oxygenase Stop
 TGGCAGCTGCCCACTGCCGCCCTGTCCGGCGTGGCCTGGTGCCTGGCATGA
 W Q L P H C R R L S G R G L V P A L A *

FIG.1 (cont.)

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C-4 oxygenase untranslated region Nos term
CCTGGTCCCTCCGCTGGTGACCCAGCGTCTGCACAAGAGTGTATGGAGCTCGAATTTC
CCGATCGTTCAAACATTTGGCAATAAAGTTCTTAAGATTGAATCCTGTTGCCGGTCTTG
CGATGATTATCATATAATTCTGTTGAATTACGTTAACATGTAATAATTAAACATGTAAT
GCATGACGTTATTTATGAGATGGGTTTTATGATTAGAGTCCCGCAATTATAACATTAAAT
ACCGCGATAGAAAACAAAATATAGCGCGCAAACCTAGGATAAAATTATCGCGCGCGGTGTCAT
end
CTATGTTACTAGATCGGGAATTC

Fig. 1 (cont.)

29295/BN

Abstract

A DNA construct comprising in the 5' to 3' direction of transcription operably linked a promoter region directing transcription to the seed of an oilseed plant, a nucleotide sequence 5 coding for at least one peptide with enzyme activity necessary for keto group containing xanthophyll production and esterification in an oilseed plant and a transcriptional termination region is disclosed. The DNA construct may additionally comprise a nucleotide sequence coding for a transit peptide directing the translated fusion polypeptide to the chloroplast of the oilseed plant. The peptide with enzyme activity is preferably a peptide with β -carotene C-4-10 oxygenase activity, e.g. from the alga *Haematococcus pluvialis*.

Comprised by the invention are also a transgenic oilseed plant cell, e.g. of rape, sunflower, soybean or mustard origin; transgenic oilseed plant-produced xanthophyll; transgenic oilseed plant-produced canthaxanthin; transgenic oilseed plant-produced astaxanthin; and transgenic oilseed plant-produced astaxanthin esters.

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